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Extreme Diel Fluctuations of Oxygen in Diffusive Boundary Layers Surrounding Stony Corals

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Abstract. The diffusive boundary layers surrounding sessile marine organisms have been implicated in controlling an organism's metabolism and growth. We studied boundary layers surrounding hermatypic corals by monitoring oxygen concentrations on a submillimetric scale. Oxygen concentration within the boundary layers varied from supersaturation during the day to anoxia at night, although the ambient water composition remained constant. Detailed mapping and oxygen measurements revealed diel oxygen fluctuations from supersaturation (373% air saturation) in the light to complete oxygen depletion at darkness in the massive coral *Favia favus*. Exposure to a 5-cm/s current reduced the boundary layer thickness from 2.44 mm to 1.90 mm, allowing more rapid oxygen exchange across the diffusive boundary layer. Similar patterns were found in the branching coral *Stylophora pistillata*. In massive corals, the thickness of the diffusive boundary layer was negatively correlated with the size of the polyp. We suggest that the distribution of corals in areas of differential turbulence is related to the thickness of the diffusive boundary layers surrounding them.

Introduction

Oligotrophic tropical marine waters experience relatively small diel chemical changes. Hence, coral reefs in tropical waters are exposed, on a macro-scale, to a relatively constant chemical milieu. Yet the metabolic activities of macro- as well as microorganisms may cause considerable changes in the chemical composition of their near surroundings. Diffusion limitations in the stagnant layer around the organisms may induce the development of diffusive boundary layers (DBL) in proximity to the surface of the organisms. In the marine environment, intense metabolic activity can result in niches of sharp gradients of solute concentrations in the DBLs; these have been found in benthic sediments (Gundersen and Jorgensen, 1990), over hypersaline microbial mats (Revsbech *et al.*, 1983; Jorgensen and Des Marais, 1990), in planktonic organisms such as Foraminifera (Jørgensen *et al.*, 1985), and in marine snow (Alldredge and Cohen, 1987).

Diffusive boundary layers, their development, and their relationships with momentum boundary layers have been extensively studied (Revsbech and Jorgensen, 1976; Jørgensen and Des Marais, 1990; Patterson, 1992a). In seawater, most biologically important small molecules and ions such as O_2, CO_2, and Ca^{++} have a similar Schmidt number (Sc) of about 500 (Patterson *et al.*, 1991; Patterson, 1992b). Schmidt number is correlated to the ratio between the thickness of the DBL (d) and that of the momentum boundary layer (δ) as $d/\delta = Sc^{-0.33}$. Because the thickness of the momentum boundary layer over a given surface at a given flow does not depend on the solute measured, the thicknesses of the DBLs for these metabolically important small molecules are very similar (Patterson, 1992b). Therefore, by measuring the concentration profile of one of these solutes, one can estimate the DBL thickness for the others. The development of O_2 microsensors caused researchers to favor O_2 concentration measurements, occasionally accompanied by other measurements such as pH (Jorgensen *et al.*, 1985; Alldredge and Cohen, 1987) or N_2O concentration (Revsbech *et al.*, 1988).

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Like many other sessile marine organisms, corals are oxyconformers; they lack the ability to actively ventilate their external surface and thus enhance the exchange of solutes with their surroundings (Shick, 1990; Patterson, 1992a). However, unlike most other animals, hermatypic corals have endosymbiotic algae. Through the activity of these symbionts, hermatypic corals experience O_2 and CO_2 fluxes resembling those known from free-living algae. The balance between photosynthesis by the symbiotic algae and respiration by animal and algae, both occurring within the coral tissue, causes shifts from net efflux to net uptake of O_2 and CO_2. Uptake and excretion of other solutes such as Ca^{+2} and NH_4^+ occur in both passive and active transport (Crossland and Barnes, 1974; Burris, 1983; D'Elia and Cook, 1988). While studying the dynamics of solute exchange, the resistance of the water within the DBL must be taken into account. In freshwater amphibians, the DBL resistance was found to be similar to or greater than that of the animal skin (Pinder and Feder, 1990; Feder and Booth, 1992). Therefore, the thickness of the DBL may control the flux rates of solutes between the coral and its surrounding (Patterson *et al.*, 1991; Patterson, 1992b).

In hermatypic corals, high rates of solute exchange are limited by the coral's external skeleton to the direction of the open water. Thus it is possible to map and study the DBL surrounding the coral polyp (the individual module of the colony) on a two-dimensional scale. Hence, corals present a good, though complicated, model for study of the effects of structure and changes in the DBL on the metabolism of sessile marine animals and plants. By correlating DBL changes to the activity of other members of the coral community, and to environmental factors such as exposure to light and water flow, we may improve our understanding of the adaptions and distribution patterns sessile marine organisms employ to cope with these metabolic confinements.

Materials and Methods

Corals were collected from a coral reef in front of the H. Steinitz Marine Biology Laboratory, in the Gulf of Eilat, Red Sea, from depths of 5–7 m. At 6 m depth, maximal light intensity (measured in microEinsteins, where 1 μE = 1 μmol of photons) reached 980 $\mu E \cdot m^{-2} \cdot s^{-1}$. Coral colonies (up to 15 cm in diameter) and surrounding substrate were cut and transferred to the laboratory. Corals were submerged in seawater throughout their handling. After collection, corals were placed in experimental aquaria and held in position by attaching the surrounding substrate to a glass base. Corals were set in running seawater for 6 h and then aerated for an additional 3 h. Aeration was maintained throughout the experiments.

Oxygen concentrations were measured using combined oxygen microelectrodes with sensing tips 10–200 μm in diameter (Revsbech and Jorgensen, 1976). The extremely low oxygen consumption by these electrodes enabled accurate measurements to be made without stirring the water. Microelectrodes were calibrated against seawater flushed to equilibrium with nitrogen, air, or oxygen. Oxygen concentrations in the calibration chambers were determined using the Winkler-Iodometric method (Taras *et al.*, 1971). Oxygen concentration profiles were measured perpendicular to the coral tissue at vertical intervals of 0.10–0.25 mm. DBL thickness was determined as the distance at which oxygen concentration differed from that of the external water mass by more than 10% (Jørgensen and Des Marais, 1990). Oxygen profiles through the diffusion boundary layers were measured in several species of stony corals, followed by detailed measurements of oxygen profiles in the massive coral *Favia favus* and the branching coral *Stylophora pistillata*. With these two coral species, series of vertical oxygen profiles were measured in the light and in the dark, at horizontal intervals of 0.1–0.5 mm, and contour maps of oxygen concentrations within the DBL were constructed.

To evaluate the effect of water motion on the DBL, a 5-cm/s current was created in the aquaria by using a magnetic stirrer. Current velocity was measured at a distance of 1 cm perpendicular to the oxygen measurement point by tracing small suspended particles. This flow velocity is the average velocity of the strong currents measured off the laboratory in Eilat, excluding wave currents (A. Genin, pers. comm.). Oxygen profiles were measured when corals were exposed to this current in the dark. Contour maps of oxygen concentration were constructed from these profiles as well.

To define oxygen concentration within the coral skeleton, a hole 1 mm in diameter was drilled 5 cm into the

Table I

Thickness of oxygen diffusive boundary layer (DBL) in stony corals

Coral species	DBL thickness (mm)	Polyp width (mm)
Massive		
Platygyra lamellina	4.00	>10*
Favia favus	2.44	8.8 ± 1.0
Favites pentagona	1.75	8.7 ± 1.6
Porites lobata	1.50	1.9 ± 0.3
Branching		
Stylophora pistillata	3.09	0.9 ± 0.1

DBL thickness was determined as the distance, perpendicular to the opening of the polyps' oral disk, in which oxygen concentration differed from that in the external water mass by more than 10% (Jorgensen and Des Marais, 1990). Measurements were conducted in the dark and in calm seawater.

* *Platygyra lamellina* is a brain coral, and the size of the opening over the polyps increases as the coral grows.

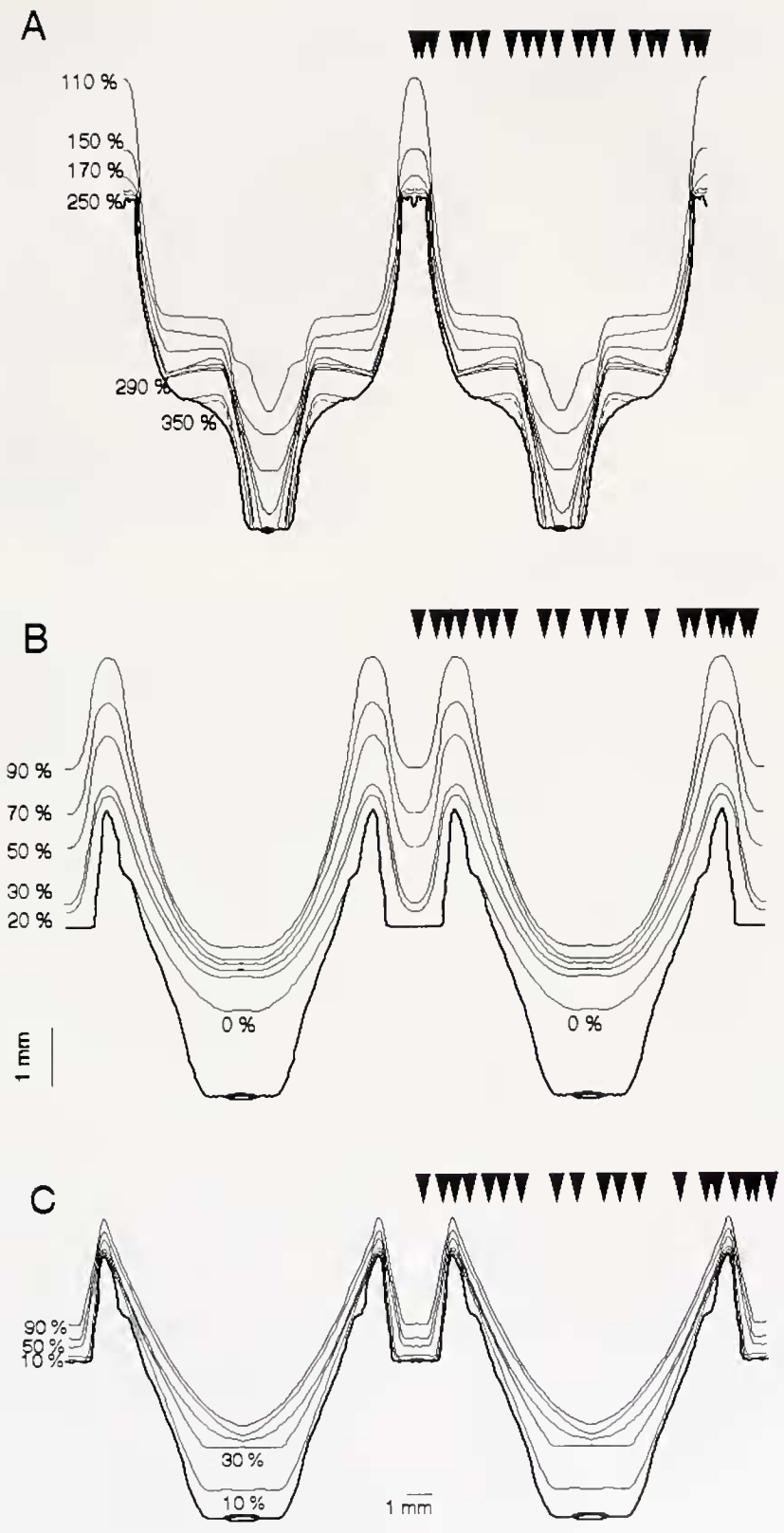
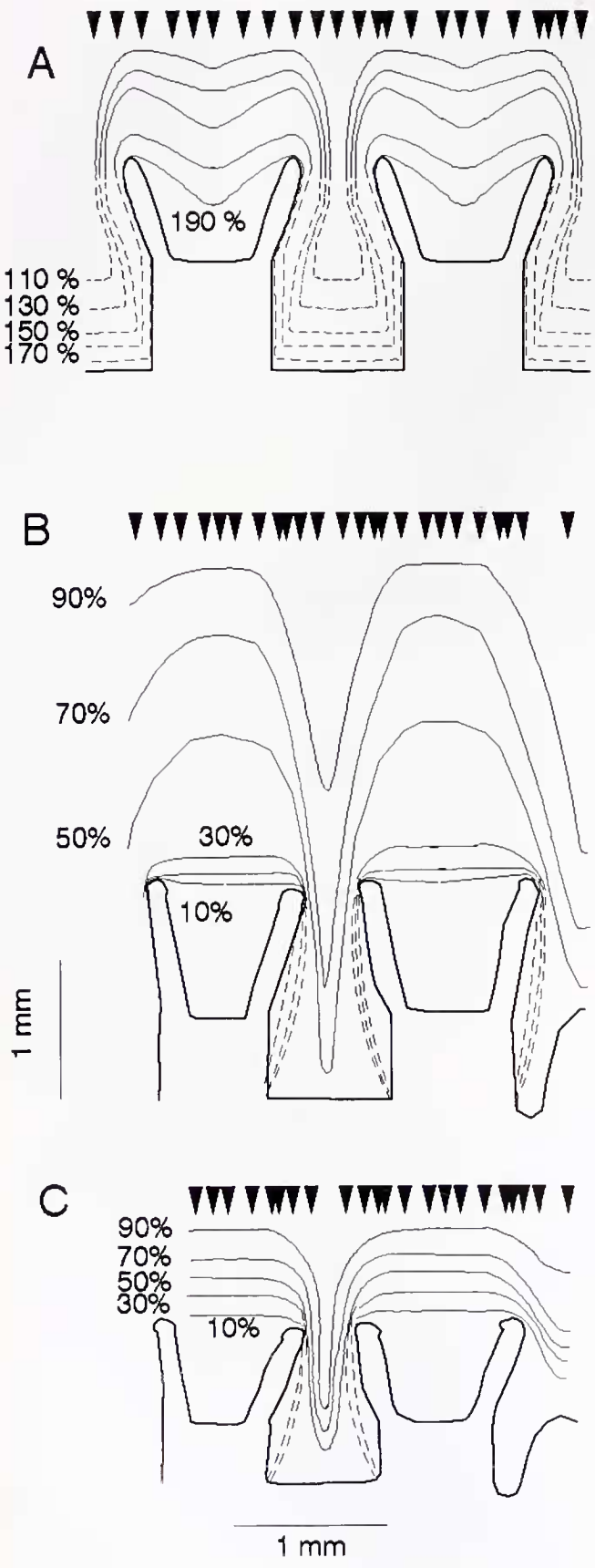


Figure 1. Contour maps of the oxygen boundary layers surrounding *Favia favus* coral polyps. Each map was constructed from measurements of oxygen profiles taken from 10 different colonies. Arrows indicate points of profile measurements. Tissue surface is marked in bold lines. Dashed lines mark estimated values where continuous measurements were not possible. For better illustration, maps were duplicated by left symmetry. (A) Oxygen boundary layer under light conditions of 980 $\mu E \cdot m^{-2} \cdot s^{-1}$. (B) Oxygen boundary layer in darkness. (C) Oxygen boundary layer under a 5-cm/s current, in darkness.



skeleton of a living *F. favus* coral. The hole was cleaned with a gentle water jet. The oxygen concentration was measured within the hole in total darkness, 1 h after the light was turned off.

To evaluate the rate of oxygen production, oxygen concentration was monitored at the opening of a polyp of *S. pistillata* coral during shifts from light to darkness. The microelectrode was positioned 0.5 mm perpendicular to the opening of the oral disk, and between the coral tentacles, for at least 5 min. in the light. The light was not turned off until the tentacles were extended and not affected by the electrode. Oxygen concentration was recorded continuously.

Light intensity, provided by a Scott KL-1500 light source equipped with a fiber optic, was 980 $\mu E \cdot m^{-2} \cdot s^{-1}$ in all experiments. Light was measured using a Li-Cor Li-1000 light meter with an underwater, cosine corrected, 2π sensor.

Results

A defined oxygen DBL was identified in all stony corals examined (Table I). In darkness and in calm seawater, the thickness of the oxygen boundary layers, as measured perpendicular to the opening of the polyp's oral disk, varied from 4.00 mm in *Platygyra lamellina* to 1.50 mm in *Porites lobata*. In the massive corals tested, corals having a larger calyx tended to have a thicker DBL (Table I).

Detailed measurements on *F. favus* and *S. pistillata* yielded the contour maps presented in Figures 1 and 2. These measurements revealed the existence of diffusive boundary layers completely encompassing each coral. Oxygen depletion was extreme during darkness, when oxygen concentration dropped to 10% of air saturation (22 μM) in *S. pistillata* (Fig. 2,B) and to total anoxia in *F. favus* (Fig. 1,B). When corals were exposed to a light intensity of 980 $\mu E \cdot m^{-2} \cdot s^{-1}$, supersaturation of oxygen was measured at 191% air saturation (420 μM) in *S. pistillata* (Fig. 2,A) and 373% air saturation (820 μM) in *F. favus* (Fig. 1,A). In the smaller polyped coral *S. pistillata*, the DBL extended into the surrounding water beyond the polyp tentacles.

Even in a current of 5 cm/s, a DBL was continuously present (Figs. 1 and 2,C). Though not changing the minimal oxygen concentration at the surface of the coral tissue (anoxia in *F. favus* and 10% of air saturation in *S. pistillata*), exposure to this current reduced the thickness of the DBL (Fig. 3) from 2.44 mm to 1.90 mm in *F. favus*

Figure 2. Contour maps of the oxygen boundary layers surrounding *Stylophora pistillata* coral polyps. Each map was constructed from measurements of oxygen profiles taken from 10 different colonies. Further details are as in the legend to Figure 1 (symmetry duplication was not used).

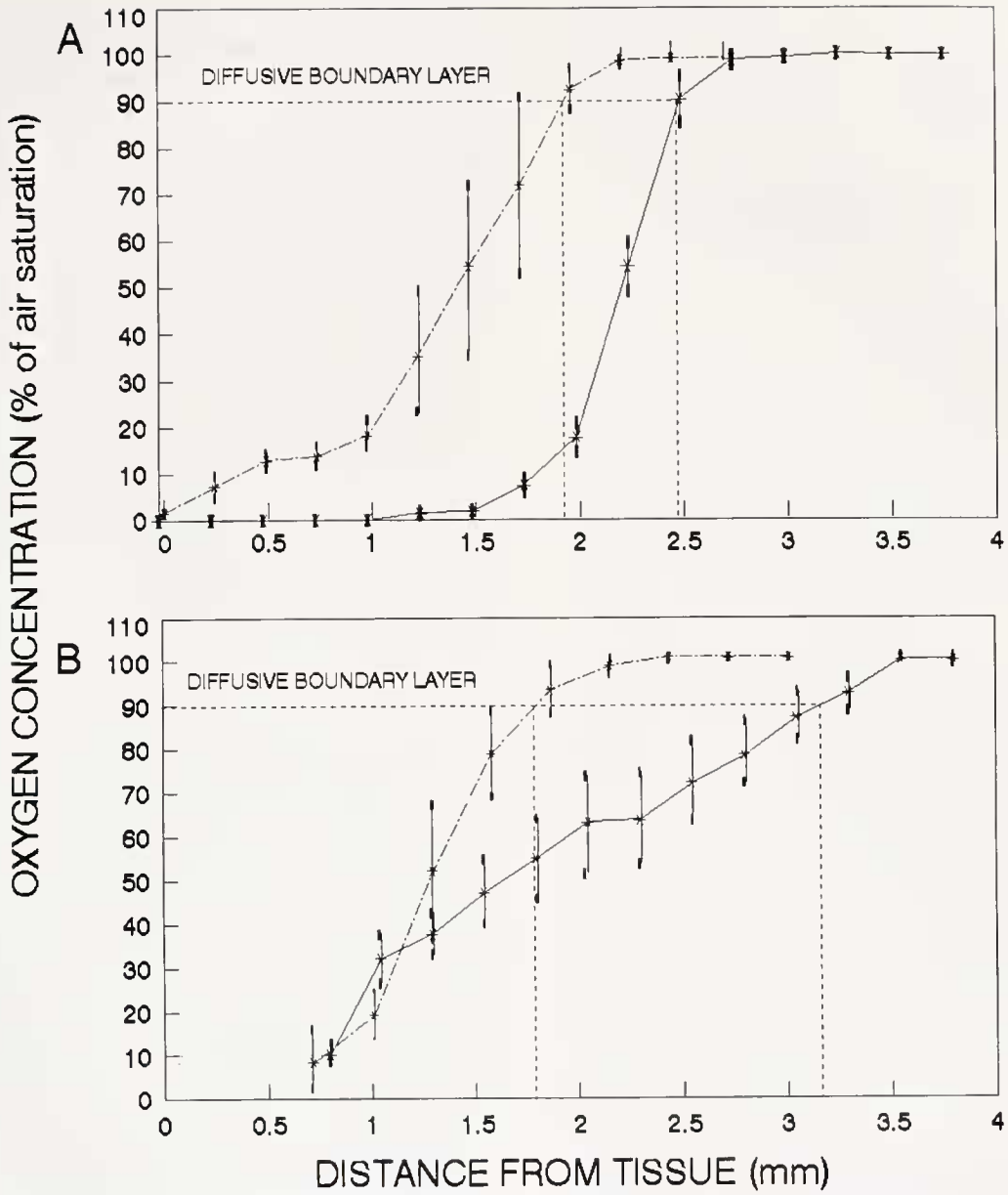


Figure 3. Oxygen concentrations perpendicular to the oral disk opening of the corals *Favia favus* (A) and *Stylophora pistillata* (B) in calm water (continuous lines) and with a 5-cm/s current (dashed lines). Means ± SD; *n*: A-calm = 4, A-current = 3, B-calm = 5, B-current = 7.

and from 3.09 mm to 1.80 mm in *S. pistillata*. Although the 5-cm/s current reduced the thickness of the DBL, it remained laminar and the iso-oxic profiles in the boundary layers remained continuous.

Measurements made in a hole drilled into the coral skeleton of an *F. favus* coral showed that, in the dark, hypoxia (13% air saturation) extended throughout the entire depth of the hole, and a defined DBL was present outside it.

When corals were shifted from light to darkness, oxygen concentrations within the diffusive boundary layer changed from supersaturation to anoxia within less than 1 min. From the initial slope of the decline in oxygen concentration, we calculated the oxygen residence time (the time needed for consumption of the excess oxygen, reducing the concentration from its level in the light to its level in the surrounding seawater, in the dark) to be 4.3 s (Fig. 4).

Discussion

Like other sessile organisms, corals create a distinct microhabitat considerably different from the water sur-

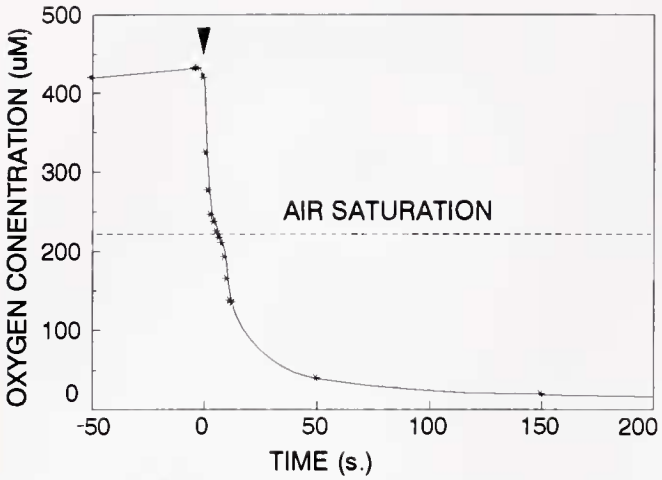


Figure 4. Changes in oxygen concentration measured between the tentacles, at a 0.5-mm distance perpendicular to the opening of the polyps' oral disk, of a *Stylophora pistillata* coral, as it was shifted from light to darkness (arrow indicates light turned off).

rounding them (Schiller and Herndl, 1989). The diffusive boundary layers describe some of the unique properties of this microhabitat. In these layers, oxygen concentrations fluctuate from supersaturation during the day to hypoxia during the night.

Two processes regulate oxygen concentration: oxygenic photosynthesis by the corals' symbiotic zooxanthellae and by endolithic photosynthetic organisms, and oxygen consumption due to respiration of the entire coral head community. The short time scale during which changes in oxygen concentrations occur indicates that the light regime is the main factor affecting the balance between respiration and photosynthesis, and thus dictating the oxygen concentration close to the coral. Other processes such as tentacle movement and water exchange within the polyp require a longer time, and were not found to be directly coupled to the fluctuations in oxygen concentration. In the field, oxygen supersaturation may develop soon after sunrise, and oxygen depletion may occur soon after sunset. Therefore, corals and endolithic algae must be adapted to function at both very low and very high oxygen concentrations. High specific activities of the enzymes superoxide dismutase, catalase, and ascorbate peroxidase have been found in isolated zooxanthellae, in coral host tissue, and in endolithic algae (Shick and Dykens, 1985; Lesser and Shick, 1989; Matta and Trench, 1991; Shashar and Stambler, 1992). These enzymes may protect the organisms against the toxic effects of elevated oxygen concentrations. In addition to possible toxic effects, high oxygen concentrations from photosynthesis will increase host respiration (Shick, 1990) and may reduce photosynthetic rates in algal symbionts. The high oxygen concentrations are expected to be coupled with CO_2 depletion and high pH levels (Shashar and Stambler, 1992). Both result in lower rates of net carbon photoassimilation. The enzyme carbonic anhydrase, found in the animal tissue and in the symbiotic zooxanthellae (Weis *et al.*, 1989), may partly reduce the enhanced photorespiration.

At night, corals are exposed to extreme internal oxygen depletion. During that time, they extend their tentacles into the water column. As has been suggested for several cnidarians (Shick *et al.*, 1979; Shick, 1990; Patterson, 1992b), the extended tentacles, by increasing the polyps' surface-to-volume ratio and exposed surface area, can enhance diffusion rates across the DBL and reduce oxygen limitation during darkness.

Currents considerably reduced the thickness of the diffusive boundary layer, although the DBL remained continuous, surrounding the surfaces of the entire coral. The thickness of the DBL for most small metabolically important molecules and ions is very similar (Patterson, 1992b). Therefore, the effect of currents on the oxygen DBL should be analogous to their effect on the DBLs of other small molecules such as CO_2 and Ca^{++}. In stony corals, diffusion rates of CO_2, O_2 and Ca^{++} have been postulated to limit photosynthesis, respiration, and calcification, respectively (Kanwisher and Wainwright, 1967; Weis *et al.*, 1989). The flux rates of oxygen and other solutes through the boundary layer can be expressed by the steepness of their concentration gradients. Higher current velocities allow more rapid solute exchange through the thinner diffusive boundary layer, probably supporting higher rates of respiration, photosynthesis, calcification, and overall growth (Jokiel, 1978; Dennison and Barnes, 1988; Patterson and Sebens, 1989).

The boundary layers can affect other organisms living within the coral head (Vogel, 1981; Atkinson, 1992). Eukaryotic endolithic algae found in stony corals survive by maintaining an overall low metabolic rate and utilizing enzymes such as catalase and carbonic anhydrase (Shashar and Stambler, 1992). Boring macroorganisms may avoid severe fluctuations in oxygen concentration by creating strong microcurrents that can alter the boundary layer in the vicinity of the boring animal. Microcurrents of 1.9–4.0 cm/s have been measured at the exhalent siphon of the boring bivalve *Lithophaga lessepsiana* (Y. Loya and J. R. Strickler, unpublished data). Because *L. lessepsiana* heavily infects the coral *S. pistillata*, these microcurrents may significantly reduce the effect of the boundary layer on both coral and bivalve.

The effect of the boundary layer on the coral and its inhabitants may in turn determine the coral reef community structure. A thick DBL may limit a coral's tolerance to low current areas or to extreme light intensities. In comparing the thickness of DBLs in large-polyped and small-polyped corals, our results sustain Patterson's (1992a) assumption that the thickness of the DBL is pro-

portional to organism size. We predict that small-polyped corals, with thinner diffusive boundary layers, will be more abundant in reef habitats characterized by low currents, whereas large-polyped corals will be more abundant in reef areas of higher turbulence. The community structure and zonation patterns in Eilat support this prediction (Loya and Slobodkin, 1971; Loya, 1972). As examples, the small-polyped coral *Porites lutea* is the primary species at a depth of 30 m, where the water is relatively calm; and the large-polyped *Platygyra lamellina* is most abundant and has the largest colony size at depths less then 3 m, where the water is more turbulent.

Corals grow under various light and flow conditions. This wide range of external conditions combines with specific behavior such as extension of tentacles, secretion of mucus, and creation of local microcurrents to shape the boundary layer surrounding the colony. Further studies of solute transport mechanisms through the DBL in other sessile organisms, as well as more information about the exact reactions of different coral species to diffusion limitations, are needed to increase our understanding of the ways sessile aquatic organisms cope with their extremely fluctuating environment.

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Literature Cited

Alldredge, A. L., and Y. Cohen. 1987. Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets. *Science* **235:** 689–691.

Atkinson, M. J. 1992. Productivity of Enewetak Atoll reef flats predicted from mass transfer relationships. *Continental Shelf Res.* **12**(7/8): 799–807.

Burris, R. H. 1983. Uptake and assimilation of $^{15}NH_4$ by a variety of corals. *Mar. Biol.* **75:** 151–155.

Crossland, C. J., and D. J. Barnes. 1974. The role of metabolic nitrogen in coral calcification. *Mar. Biol* **28:** 325–332.

D'Elia, C. F., and C. B. Cook. 1988. Methylamine uptake by zooxanthellae-invertebrate symbioses: insights into host ammonium environment and nutrition. *Limnol. Oceanogr.* **33**(5): 1153–1165.

Dennison, C. W., and D. J. Barnes. 1988. Effect of water motion on coral photosynthesis and calcification. *J. Exp. Mar. Biol. Ecol.* **115:** 67–77.

Feder, M. E., and D. T. Booth. 1992. Hypoxic boundary layer surrounding skin-breathing aquatic amphibians: occurrence, consequences and organismal responses. *J. Exp. Biol.* **166:** 237–251.

Gundersen, J. K., and B. B. Jørgensen. 1990. Microstructure of diffusive boundary layers and the oxygen uptake of the sea floor. *Nature* **345:** 604–607.

Jokiel, P. 1978. Effects of water motion on coral reefs. *J. Exp. Mar. Biol. Ecol.* **33:** 87–97.

Jørgensen, B. B., J. Erez, N. P. Revsbech, and Y. Cohen. 1985. Symbiotic photosynthesis in the planktonic foraminiferan *Globigerinoides sacculifer* (Bardy), studied with microelectrodes. *Limnol. Oceanogr.* **30**(6): 1253–1267.

Jørgensen, B. B., and D. J. Des Marais. 1990. The diffusive boundary layer of sediments: oxygen microgradients over a microbial mat. *Limnol. Oceanogr.* **35**(6): 1343–1355.

Kanwisher, J., and S. A. Wainwright. 1967. Oxygen balance in some reef corals. *Biol. Bull.* **133:** 378–390.

Lesser, M. P., and J. M. Shick. 1989. Photoadaptation and defenses against oxygen toxicity in the zooxanthellae from natural populations of symbiotic cnidarians. *J. Exp. Mar. Biol. Ecol* **134:** 129–141.

Loya, Y. 1972. Community structure and species diversity of hermatypic corals at Eilat, Red Sea. *Mar. Biol.* **13:** 100–123.

Loya, Y., and L. B. Slobodkin. 1971. The coral reefs of Eilat (Gulf of Eilat, Red Sea). *Symp. Zool. Soc. Lond.* **28:** 117–139.

Matta, J. L., and R. K. Trench. 1991. The enzymatic response of the symbiotic dinoflagellate *Symbiodinium microadriaticum* (Freudenthal) to growth *in vitro* under varied oxygen tensions. *Symbiosis* **11:** 31–45.

Patterson, M. R. 1992a. A mass transfer explanation of metabolic scaling relations in some aquatic invertebrates and algae. *Science* **255:** 1421–1423.

Patterson, M. R. 1992b. A chemical engineering view of cnidarian symbioses. *Am. Zool.* **32:** 566–582.

Patterson, M. R., and K. P. Sebens. 1989. Forced convection modulates gas exchange in cnidarians. *Proc. Natl. Acad. Sci. U. S. A.* **86:** 8833–8836.

Patterson, M. R., K. P. Sebens, and R. R. Olson. 1991. *In situ* measurements of flow effects on primary production and dark respiration in reef corals. *Limnol. Oceanogr.* **36:** 936–948.

Pinder, A. W., and M. E. Feder. 1990. Effect of boundary layers on cutaneous gas exchange. *J. Exp. Biol.* **154:** 67–80.

Revsbech, N. P., and B. B. Jørgensen. 1976. Microelectrodes: their use in microbial ecology. *Adv. Microb. Ecol.* **9:** 293–352.

Revsbech, N. P., B. B. Jørgensen, T. H. Blackburn, and Y. Cohen. 1983. Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles of a microbial mat. *Limnol. Oceanogr.* **28**(6): 1062–1074.

Revsbech, N. P., L. P. Nielsen, P. B. Christensen, and J. Sorensen. 1988. Combined oxygen and nitrous oxide microsensor for denitrification studies. *Appl. Environ. Microbiol* **54**(9): 2245–2249.

Schiller, C., and G. J. Herndl. 1989. Evidence of enhanced microbial activity in the interstitial space of branched corals: possible implications for coral metabolism. *Coral Reefs* **7:** 179–184.

Shashar, N., and N. Stambler. 1992. Endolithic algae within corals—life at an extreme environment. *J. Exp. Mar. Biol. Ecol.* **163:** 277–286.

Shick, J. M. 1990. Diffusive limitation and hyperoxic enhancement of oxygen consumption in zooxanthellae, sea anemones, zoanthids and corals. *Biol. Bull.* **179:** 148–158.

Shick, J. M., and J. A. Dykens. 1985. Oxygen detoxification in algal—invertebrate symbioses from the Great Barrier Reef. *Oecologia* **66:** 33–41.

Shick, J. M., W. I. Brown, E. G. Dolliver, and S. R. Kayar. 1979. Oxygen uptake in sea anemones: effects of expansion, contraction, and exposure to air and the limitation of diffusion. *Physiol. Zool.* **52**(1): 50–62.

Taras, M. J., A. E. Greenberg, R. D. Hoak, and M. C. Rand. 1971. Pp. 477–481 in *Standard Methods for Examination of Water and Waste Water.* American Public Health Ass.

Vogel, S. 1981. Pp. 152–153 in *Life in Moving Fluids.* Willard Grant Press. Boston.

Weis, V. M., G. J. Smith, and L. Muscatine. 1989. A CO_2 supply mechanism in zooxanthellae cnidarians: role of carbonic anhydrase. *Mar. Biol.* **100:** 195–202.